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GROWTH AND REGENERATION PATTERNS IN THE FIDDLER CRAB, *UCA PUGILATOR*

PENNY M. HOPKINS

Department of Zoology, 730 Van Vleet Oval, University of Oklahoma, Norman, OK 73019

ABSTRACT

The fiddler crab, *Uca pugilator*, will survive several intermolt cycles in the laboratory, but the cycles are irregular. Variations in cycles are due to variations in the length of stage C_4. The transition from C_4 to D in intact crabs does not seem to be due to environmental clues because crabs kept in constant conditions for long periods of time continue to have extremely variable intermolt cycles.

Multiple autotomy triggers the onset of proecdysis and a post-autotomy intermolt cycle that is significantly shorter than controls. Multiple autotomy-induced proecdysis is divided into two phases: the "reset event" is independent of the eyestalks, while the "proecdysial program" is normally under their control. Loss of a cheliped is more effective in initiating a reset event than is loss of a single walking leg.

Eyestalk removal forces crabs into proecdysis. If crabs are in early proecdysis (stage D_0) at eyestalk removal, the proecdysial period is accelerated. Eyestalk removal results in large increases in size at ecdysis which can be blocked by multiple autotomy. Ecdysis does not always result in growth. Molting in *Uca* may result only in regeneration of missing limbs. Crabs regenerating a number of limbs may actually become smaller at molt.

INTRODUCTION

Ecdysis of the calcified exoskeleton is the end point of a combination of physiological processes used by decapod crustaceans to achieve both general body growth and regeneration of appendages. Implicit in this statement is the assumption that the controls of ecdysis, growth, and regeneration are intimately linked and finely coordinated. Ecdysis and regeneration can be induced during non-growth periods by removal of the eyestalks or of many appendages. The former method induces ecdysis through removal of inhibitory neurosecretory centers in the eyestalk (the x-organ and sinus gland). The removal of the inhibitory centers usually causes a premature ecdysis. The second type of molt induction (called multiple autotomy) is more complicated and is thought to involve a "resetting" of the physiological processes that culminate in regeneration and ecdysis (Skinner and Graham, 1972).

The fiddler crab, *Uca pugilator*, is a durable and exceptional laboratory animal. One of the most remarkable features of these hardy little crabs is the single, large cheliped of the male (from which this entire group gets its common name). This cheliped (or claw) is often longer than the entire carapace of the crab. One third of the wet weight of a male crab may be due to the cheliped. The cheliped is very important in social and reproductive behavior of these crabs (Crane, 1975).

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Abbreviations: C_4, intermolt period of molt cycle; D, proecdysial period of molt cycle; E, ecdysis; ER, experimental growth rate; MA, multiple autotomy (including cheliped); MA-CI, multiple autotomy (cheliped intact); R_3, right third walking leg; R-value, regeneration index value for R_3.

The fiddler crab has been used by many investigators in various physiological and endocrinological studies (Abramowitz and Abramowitz, 1940; Guyselman, 1953; Passano, 1960; Vernberg and O'Hara, 1972; Skinner and Graham, 1972; Fingerman and Fingerman, 1974; Weis, 1976, 1977a,b). Relatively little has been reported, however, concerning growth and molt cycles of intact animals under constant laboratory conditions. This paper describes the molting cycles in intact fiddler crabs kept in constant environmental conditions and compares these "normal" cycles to autotomy-induced and eyestalk removal-induced cycles.

This paper includes observations on: (1) the effect of autotomy of various numbers of limbs upon the "reset" and duration of the intermolt cycle and growth patterns of the carapace and limbs; (2) the influence of autotomy of the large cheliped upon the intermolt cycle; (3) the effects of autotomy and eyestalk removal upon intermolt cycles subsequent to the induced cycles. The seemingly contradictory effects of multiple autotomy upon eyed and eyestalkless crabs has been investigated and a modified model for autotomy-induced proecdysis is proposed.

Materials and Methods

Male specimens of the fiddler crab, *Uca pugilator*, were obtained from the Gulf Specimen Company of Panacea, Florida. Shipments were received throughout the year. Upon arrival in the laboratory, the crabs were forced to autotomize the right third (R_3) walking leg (the fourth pereiopod) by pinching the merus with forceps. Individual crabs were kept in transparent plastic boxes (28 cm × 17.5 cm × 13.5 cm) with a small amount of artificial sea water (Instant Ocean, Aquarium Systems, Inc., Menton, Ohio). Crabs were kept in environmental chambers maintained at 23°C with 12 hours of illumination each day beginning at 6:00 AM. The crabs were fed oatmeal once a week and allowed to feed overnight. The water in the boxes was changed the following day. Animals were checked daily for molts. Crabs were allowed to acclimate in the laboratory at constant environmental conditions for at least two weeks prior to being used in any experiment.

The carapace width of each animal was measured with a vernier caliper (Moderntools, MT-9). The regenerating right third walking leg was measured every other day with the aid of an ocular micrometer in a dissecting microscope. In order to compare limbs from crabs of different carapace size, the length of a regenerating limb bud was converted to a Regeneration Index (Bliss, 1956).

$$\text{Regeneration Index (R-value)} = \frac{\text{length of limb bud (in mm)}}{\text{carapace width (in mm)}} \times 100$$

Subdivisions or stages of intermolt cycles were assigned as per Skinner (1962, after Drach, 1939).

The length of the large cheliped was also measured with the vernier caliper. This measurement is the linear distance from the notch at the base of propodus (at the point of articulation with the carpus) to the tip of the dactylus. The size of the cheliped (in mm) was divided by the carapace width (in mm) and this number is called the "Cheliped/Carapace Ratio" (C/C Ratio).

Following the emergence of the right third limb bud, multiple autotomy of additional walking legs and/or the large cheliped was induced as described above for the right third walking leg.

Eyestalks were removed by cutting the articulating membrane with a pair of dissecting scissors. Prior to eyestalk removal, animals were anesthetized by cooling at 4°C for 10 to 20 minutes.

Growth rates (ER's) of regenerating limb buds were calculated as previously described (Bliss and Hopkins, 1974): R_3 values are plotted against time (in days). For two consecutive R_3 values, the slope of the line connecting the points is taken as the experimental growth rate (ER) for the limb. The slope of the line is the arc angle of the sloped line relative to the horizontal. ER's were calculated for every day of an intermolt cycle. The average ER is the mean of those daily ER's.

Water content of chelipeds and walking legs was determined by blotting and weighing the limb immediately after removal, desiccating in a drying oven for four to six days, then weighing again. The difference in weight was taken as the water content of the limb. Protein content of chelipeds and walking legs was determined by grinding the desiccated limbs in ice cold 5% trichloroacetic acid (TCA) in a chilled mortar and pestle. The solution was centrifuged at 4°C and 10,000 × *g* for 20 minutes. The pellet was re-extracted with successive extractions in 80% and 100% ethanol, chloroform:ether (2:1 vol:vol), and ethyl ether. The pellet was resuspended in distilled water and the amount of protein determined by the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma Chemical Co.) as the standard.

Statistical analysis of the data was handled as follows: means were determined and the homogeneity of variances was tested using the F_{max} test (Sokal and Rohlf, 1969, p. 370). If the assumptions for normality were met, analyses were done using standard analysis of variance. However, if the assumptions of analysis of variance were not met, analogous non-parametric methods (Mann-Whitney U-test and Wilcoxon two-sample test) were used.

Results

Intermolt cycles in control animals

The duration of intermolt cycles in intact crabs varies from crab to crab and from cycle to cycle. When maintained under the constant laboratory holding conditions described above, *Uca pugilator* can successfully complete as many as six intermolt cycles (Table I and Fig. 1a). The durations of these cycles range from 25 to 171 days. For crabs kept in the lab over three months, the range is 34 to 136 days. Animals maintained under constant environmental conditions for several months show some reduction in the mean duration of the intermolt cycles. The eventual clustering around a mean intermolt cycle of 70 days (Table I) is the result of a reduction in the number of extremely long intermolt cycles. The number of shorter cycles is unaffected.

After being held at constant conditions for several months, however, individual crabs continue to molt independently of one another: there are no "waves" of molting. The pattern of variable intermolt durations differs from one crab to another. An individual crab may take 125 days to complete one intermolt cycle and complete the next cycle in less than 50 days. Another crab in identical holding conditions may have two very long (or very short) successive intermolt cycles (Fig. 1a). The duration of a single intermolt cycle is never a prediction of the duration of subsequent cycles.

In *Uca*, the proecdysial period of any intermolt cycle requires about 27 days (Table II). This is true for crabs missing only one limb and for crabs missing eight limbs. Crabs that are destalked during C_4 (see Drach, 1939) take 26.6 days to reach ecdysis. Thus, the variations observed in intermolt cycle lengths represent variations in the duration of stage C_4 rather than in stage D.

These differences between cycle durations of crabs that have been in the lab under identical conditions are, in part, due to the variations in the sizes of the

TABLE I

Number of days (mean ± standard error of the mean) from initial event (either autotomy of a single R_3 walking leg, multiple autotomy or eyestalk removal) to the first ecdysis in the lab.

	Controls (Lacking a single R_3)		Multiple autotomy: 8 Walking legs (MA-CI)		Multiple autotomy: Cheliped Intact		Multiple autotomy: 7 Walking legs + cheliped (MA)		Eyestalkless	
	Number of days (±SEM)	n	Number of days (±SEM)	n			Number of days (±SEM)	n	Number of days (±SEM)	n
Initial event to Ecdysis 1	98.2 (±5.2)	75	26.0 (±1.1)	23			32.4 (±0.9)	89	22.7 (±0.8)	145
Ecdysis 1 to Ecdysis 2	85.0 (±4.2)	53	58.4 (±8.0)	11			67.0 (±3.9)	54	27.4 (±1.2)	28
Ecdysis 2 to Ecdysis 3	69.4 (±5.0)	27	79.3 (±19.0)	8			64.5 (±6.4)	19	28.0 (±1.4)	2
Ecdysis 3 to Ecdysis 4	70.2 (±7.3)	17	92.2 (±15.2)	6			67.8 (±7.9)	9		
Ecdysis 4 to Ecdysis 5	68.8 (±7.8)	10	93.2 (±14.5)	6			40.5 (±6.9)	5		
Ecdysis 5 to Ecdysis 6	76.4 (±5.8)	10	87.7 (±7.4)	3			99.3 (±40.3)	4		

The mean number of days (±SEM) for subsequent ecdyses is also given. The number of crabs in each group is given as "n."

animals. When the duration of three subsequent intermolt cycles is plotted against the initial carapace width of the animal, a correlation of 0.43 is seen. (This correlation is significant at $P < 0.01$.) For example, a specific animal of carapace width 16.85 mm took 532 days to complete three intermolt cycles of varying durations. Whereas, a smaller crab, carapace width 14.70 mm, took only 220 days to complete three cycles. The pattern of alternating short and long intermolt cycles, however, remains the same in large and in small crabs.

Intermolt cycles following autotomy

Autotomy of a single walking leg does not markedly affect the duration of the intermolt cycle (Fig. 1a). Therefore, crabs missing only one limb are referred to as "normal" or "controls" throughout this report.

The duration (and variance) from autotomy to ecdysis decreases as the regeneration load is increased (Fig. 2 and Table III). It continues to decrease until the load reaches 7 to 8 mg of protein. The regeneration load for an animal is calculated from the total amount of protein extracted from newly regenerated limbs following ecdysis.

Multiple autotomy during intermolt cycle stage C_4 significantly hastens the next ecdysis (Fig. 1b, Tables I and II). If eight walking legs are autotomized simultaneously and the cheliped left intact (MA-CI), the length of time from autotomy (= initial event) to the induced ecdysis is significantly shortened when compared to controls (Table I). However, the addition of the large, muscular claw to the regeneration load (MA) results in a period that is significantly longer ($P < 0.001$) than the comparable period in crabs missing only eight walking legs (Tables I and II).

The influence of an autotomized cheliped upon the induction of the proecdysial period (intermolt stage D) seems to be quantitatively different from the influence of the autotomy of a single walking leg. Autotomy of four walking legs shortens the time from autotomy to ecdysis when compared to controls (Table III). However, autotomy of three walking legs and the cheliped results in a significantly faster onset of ecdysis. Multiple autotomy of seven walking legs plus the cheliped (MA) is more effective in prolonging the late proecdysial period (D_1) than multiple autotomy of eight legs only. MA prolonged late proecdysis (stage D_1) in 11 out of 13 animals, while MA-CI was effective in prolonging D_1 in only three out of eight animals (Table II).

Multiple autotomy also has a pronounced effect on the second post-autotomy intermolt cycle (Fig. 1b and Table I). Not only is the immediately induced cycle affected by MA and MA-CI but also the second post-autotomy intermolt cycle is significantly shorter than that of the controls (Table I).

Intermolt cycles following eyestalk removal

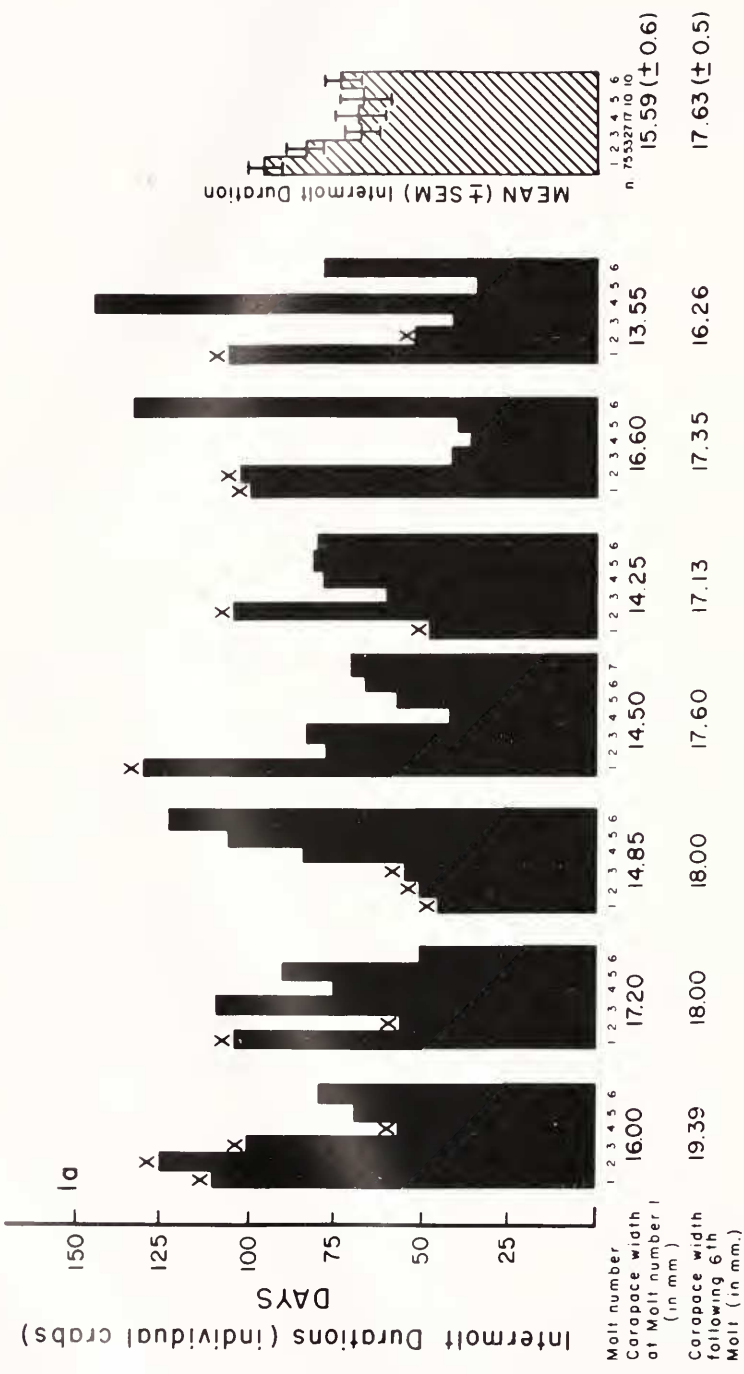
Removal of eyestalks hastens ecdysis (Tables I and II). When eyestalks are removed from crabs that have spontaneously entered proecdysis (stage D_0), the proecdysial period is shortened from 27.1 days to 18.7 days (Table II). Eyestalk removal from crabs in late proecdysis (stage D_1) reduces that period from 11.7 days to 4.8 days. Thus, it appears that the eyestalks continue to exert some inhibitory control during most of the proecdysial period. About 20% of eyestalkless *Uca* will live through a second molt cycle. These crabs molt within 28 days of the first ecdysis (Table I).

Multiple autotomy of seven legs and cheliped (MA) after eyestalk removal significantly prolongs the time from eyestalk ablation to ecdysis (Table IV). However, the number of days from MA to ecdysis (E) is less than comparable periods induced by MA in intact animals (Table IV). In fact, the time from MA to ecdysis in eyestalkless crabs is very close to (and statistically indistinguishable from) the time from eyestalk removal to ecdysis of otherwise untreated crabs (Table IV). Thus, MA in eyestalkless crabs may reset the proecdysial period but does not have any effect on the duration of the proecdysial period that follows.

Multiple autotomy during late proecdysis in eyestalkless crabs does not reset and actually speeds the proecdysial period. These crabs molt more quickly than do eyestalkless controls and they do not regenerate any of the newly autotomized limbs (Table IV).

Growth patterns in control animals

Regeneration of walking legs. The averaged growth pattern of several right third walking limb buds is illustrated in Figure 3 (solid circles and solid line). The first event in limb regeneration is emergence of a limb bud papilla through the scar tissue that covers the coxal stump. The time between autotomy of a single limb and


Intermolt Durations (individual crabs)
DAYS
150
125
100
75
50
25
Ia
Molt number
Carapace width at Molt number I (in mm)
Carapace width following 6th Molt (in mm.)
1 2 3 4 5 6
16.00
17.20
1 2 3 4 5 6
18.00
1 2 3 4 5 6
14.85
18.00
1 2 3 4 5 6 7
14.50
17.60
1 2 3 4 5 6
14.25
17.13
1 2 3 4 5 6
16.60
17.35
1 2 3 4 5 6
13.55
16.26
MEAN (± SEM) Intermolt Duration
1 2 3 4 5 6
n 7 5 3 2 7 7 10 10
15.59 (± 0.6)
17.63 (± 0.5)

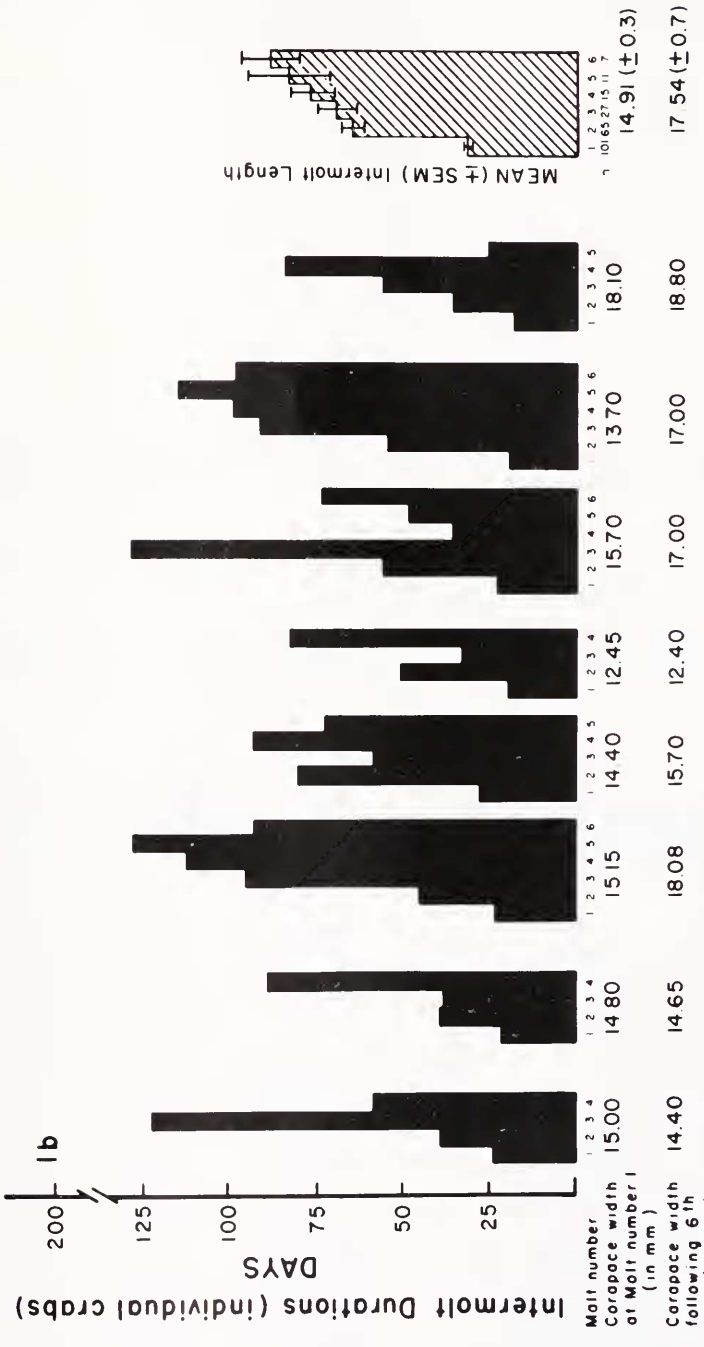


FIGURE 1. Intermolt cycle durations for selected individual crabs. (1a.) Control crabs (black bars) missing one R_3 walking leg (X) or missing no limbs (no X over bar). Means (± standard error of the mean) for large sample size (n) are given on right (lined bars). (1b.) Intermolt cycle durations of crabs missing seven walking legs plus cheliped (MA) or eight walking legs (MA-CI) prior to molt number one. Means (±SEM) for large combined samples (MA + MA-CI) are given on right (lined bars).

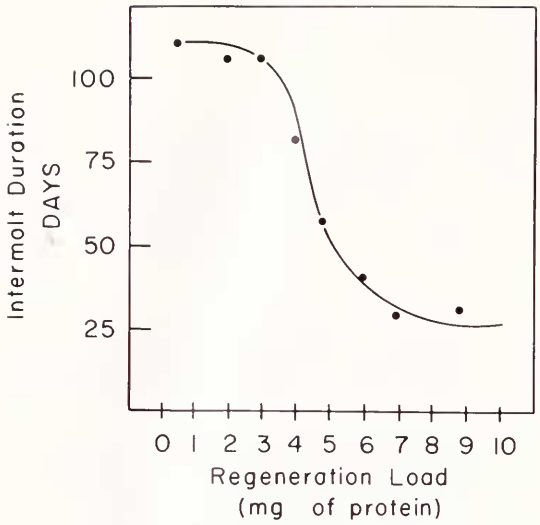


FIGURE 2. Intermolt duration (in days) as a function of regeneration load (= sum of extractable protein in mg from all regenerated limbs following ecdysis). Each point represents the mean of at least ten animals.

emergence of the papilla is quite variable in control crabs (Table III). Limb bud emergence in controls takes an average of 47% of the total cycle regardless of the length of the ensuing intermolt cycle.

The simultaneous loss of two or four walking legs hastens the emergence of all limb buds and significantly reduces the variances of emergence time (Table III).

TABLE II

The effects of multiple autotomy of eight walking legs with cheliped left intact (MA-CI), multiple autotomy of seven walking legs plus cheliped (MA), and eyestalk removal (ES) at intermolt cycle stages C_4, D_0, and D_1 on the mean number of days from treatment (T) to ecdysis (E), the final mean R_3 R-value, and overall growth rate (ER).

Treatment (T)	Molt stage at T (after Skinner, 1962)	Mean R_3 value at T, (±SEM)	Sample size (n)	Mean number of days from T to ecdysis (E), (±SEM)	Mean final R_3 value (±SEM)	Mean overall growth rate (ER) T to E, (±SEM)
Control	C_4	0	40	96.8 (±5.4)	22.50 (±0.5)	18.1 (±1.9)
MA-CI	C_4	0	14	24.9 (±2.0)	22.94 (±0.6)	42.6 (±2.3)
MA	C_4	0	20	29.7 (±2.0)	21.48 (±0.4)	35.5 (±3.7)
ES	C_4	0	42	26.6 (±1.3)	21.35 (±0.9)	39.0 (±1.6)
Control	D_0	13.11 (±0.2)	42	27.1 (±2.5)	22.42 (±0.5)	30.0 (±2.4)
MA-CI	D_0	12.80 (±0.5)	15	25.1 (±1.1)	23.20 (±0.4)	28.6 (±2.4)
MA	D_0	13.32 (±0.5)	15	28.0 (±1.5)	23.00 (±0.4)	28.0 (±2.4)
ES	D_0	12.52 (±0.4)	24	18.7 (±1.5)	22.18 (±0.4)	30.9 (±1.7)
Control	D_1	20.30 (±0.7)	27	11.7 (±2.0)	24.00 (±0.4)	20.9 (±5.6)
MA-CI	D_1	20.30 (±1.0)	3	21.0 (±0.6)	22.48 (±1.1)	9.8 (±1.7)
	D_1	23.16 (±1.3)	5	4.6 (±1.7)*	23.16 (±1.3)	0
MA	D_1	21.48 (±3.2)	11	21.6 (±1.8)	24.58 (±1.0)	8.5 (±2.0)
	D_1	22.08 (±2.2)	2	5.5 (±1.5)*	22.08 (±2.2)	0
ES	D_1	20.82 (±1.0)	6	4.8 (±1.3)	22.60 (±1.2)	12.5 (±3.0)

Means are given ± the standard error of the mean.

* MA-CI or MA limbs not regenerated.

TABLE III

Autotomy-induced reductions in means (± standard error of means) of limb bud emergence time and/or reduction in the variance ($V = (y - \bar{y})^2/n - 1$) of bud emergence and intermolt cycle durations.

Number of missing limbs (autotomized during stage C_4)	Sample size (n)	Mean number of days (±SEM)			Variances		
		Autotomy to bud emergence	Bud emergence to ecdysis	Autotomy to ecdysis	Autotomy to bud emergence	Emergence to ecdysis	Autotomy to ecdysis
1	41	43.5 (±3.6)	65.8 (±5.0)	110.4 (±5.8)	518.7	760.3	1008.0
2	19	18.0 (±2.0)	48.5 (±8.9)	72.8 (±6.7)	70.3**	719.8†	491.1
4	11	10.5 (±0.7)	46.0 (±8.7)	73.5 (±6.8)	4.2[1]	682.0††	510.8
3 + Cheliped	16	7.8 (±0.8)	40.6 (±5.0)	51.0 (±3.9)	10.3	327.8	203.1
8 (MA-CI)	37	7.7 (±0.4)	19.2 (±2.9)	28.6 (±1.6)	5.1	75.8	38.2
7 + Cheliped (MA)	35	8.7 (±0.5)	23.0 (±2.4)	33.0 (±2.3)	8.4	73.8	91.7
1*	12	8.7 (±0.7)	57.1 (±8.7)	60.1 (±8.6)	5.4	601.9	598.8

The pooled variance ratios were calculated to test the equality of variance and the variance ratio (F) was considered significant at $P < 0.05$.

Abbreviations are as in Table II.

* Autotomized following MA-induced ecdysis.

** $F = 7.4$ ($P < 0.01$).

[1] $F = 16.7$ ($P < 0.01$).

† $F = 1.06$ ($P > 0.05$).

†† $F = 1.06$ ($P > 0.05$).

Following the emergence of the limb bud papilla, a small limb bud begins to grow. This portion of limb regeneration is called basal growth (Bliss, 1956). In *Uca*, an R_3 bud will reach R-values of 10 to 13 during basal growth. Basal growth in control crabs is limited to stage C_4. The growth rate (ER) of the limb bud during this period is very slow (less than 18) and the small amount of growth that does occur may occur in discontinuous spurts.

In control crabs, rapid proecdysial growth begins at approximately 75% of the entire intermolt cycle. The ER of the limb bud may reach values of 30 to 40 (Table II). The limb bud grows and differentiates, and the muscles, chromatophores, and

TABLE IV

The effects of multiple autotomy (seven walking legs plus cheliped = MA) and eyestalk removal (ES) performed separately (ES or MA) or together (ES plus MA) on mean intermolt cycle duration (in days).

	Mean initial R_3 value (±SEM)			Mean number of days (±SEM)	
	at ES	at MA	Sample size (n)	ES to E	MA to E
ES plus MA	0	—	16	27.6 (±2.2)	—
	0	3.28 (±0.7)	8	33.3 (±1.5)	24.0 (±1.3)
	0	12.36 (±0.4)	12	36.2 (±1.4)	20.1 (±0.6)
	0	19.75 (±0.7)	10	22.0 (±1.8)	6.1 (±1.2)*
ES or MA	0	0	42 20	26.6 (±1.3)	29.7 (±2.0)
	2.49 (±0.2)	2.56 (±0.5)	14 16	33.4 (±1.9)	29.4 (±1.7)
	11.54 (±0.2)	12.59 (±0.6)	12 11	20.2 (±1.9)	27.6 (±1.7)
	20.82 (±1.0)	20.23 (±0.7)	4 11	7.5 (±1.6)	19.7 (±1.8)

* No regeneration of MA limbs.

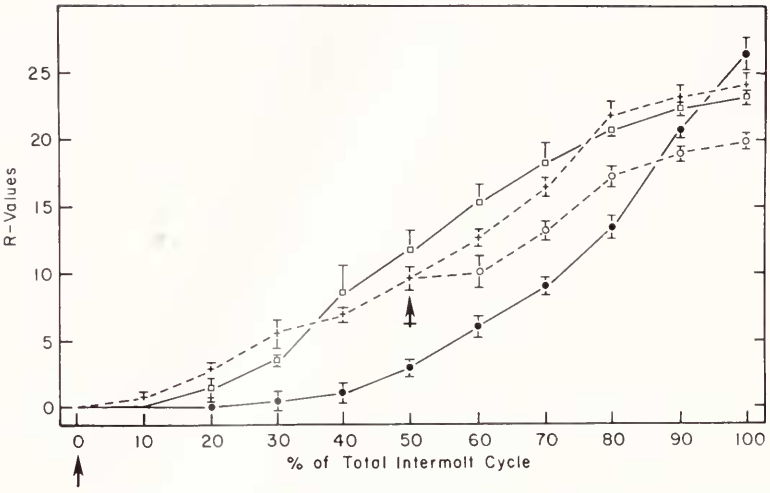


FIGURE 3. Comparison of the patterns of R_3 limb regeneration in controls (solid circles, solid line), animals missing seven walking legs plus cheliped (=MA, open squares, solid line) and eyestalkless crabs (crosses, dashed line). The arrow represents the time of MA and eyestalk ablation. The crossed arrow indicates the point at which eyestalkless crabs were forced to autotomize seven walking legs plus cheliped. The subsequent growth pattern is shown (open circles, dashed line). Each point represents the mean of at least six crabs, and the vertical lines represent standard errors of the means.

segmentation of the new limb become visible within the thin cuticle sac that covers the bud.

Regardless of the length of the intermolt cycle, the final R-values of the limb buds of control crabs are consistent (Table II). A long cycle does not result in a bigger limb bud nor does confinement to a short cycle limit the final size of the bud. A limb bud continues to grow until ecdysis. There is some "terminal plateau" (Bliss, 1956) during late proecdysis in control crabs (Fig. 3). The ER's of the limb bud are low prior to ecdysis (Table II).

At an R-value of 22 to 23, the control crabs shed the old exoskeleton. As the exoskeleton is discarded, the regenerated bud unfolds and expands. The only visible differences in a newly regenerated post-molt walking leg are its slightly smaller size and lighter color.

Carapace and cheliped growth. Following molt, the new carapace of control crabs increases in width by 2.4% (Table V). The average increase in the size of the cheliped of control crabs is 1.2%. The cheliped increases less than does carapace width. Therefore, there is a slight reduction of the cheliped/carapace ratio at each ecdysis in control crabs (Table VI). A cheliped from a control crab contains about 50% water and 7.6% protein (Table VII).

Growth patterns following autotomy

Regeneration of walking legs. Multiple autotomy not only affects the duration of the induced proecdysial period, it also has an effect upon the pattern of growth of the regenerating limb buds (Fig. 3). The limb buds of MA and MA-CI animals emerge sooner after autotomy than do the limb buds of control animals (Table III), and all of the MA and MA-CI buds emerge simultaneously. The average rate of growth (ER) of R_3 limb buds from MA crabs autotomized during stage C_4 is sig-

TABLE V

The effects of various treatments upon mean % changes in carapace and cheliped sizes and ratios.

Treatment	Sample size (n)	Mean carapace width (in mm)* (±SEM)		Mean % change (+ = increase and − = decrease) (±SEM)	Mean length of cheliped (in mm) (±SEM)		Mean % change (+ = increase and − = decrease) (±SEM)	Mean cheliped/carapace ratio (±SEM)	
		Prior to E (or to MA, or MA-CI)	After E		Prior to E (or to MA, or MA-CI)	After E		Prior to E (or to MA, or MA-CI)	After E
Controls	30	15.53 (±0.2)	15.91 (±0.2)	+2.4 (±0.2)	21.47 (±0.5)	22.12 (±0.6)	+1.2 (±0.4)	1.38 (±0.02)	1.38 (±0.03)
MA-CI	13	15.43 (±0.6)	15.45 (±0.4)	+0.12 (±0.8)	20.60 (±1.5)	20.80 (±1.4)	+1.3 (±0.7)	1.31 (±0.08)	1.36 (±0.07)
MA	17	15.24 (±0.3)	14.97 (±0.7)	−2.0 (±0.7)	20.75 (±1.5)	9.69 (±0.8)	−55.6 (±1.5)	1.41 (±0.04)	0.64 (±0.02)
2nd Post-autotomy E	16	16.19 (±0.4)	16.33 (±0.4)	+1.1 (±0.3)	11.27 (±0.6)	14.44 (±0.8)	+28.0 (±3.4)	0.69 (±0.02)	0.88 (±0.04)
Claw only missing	13	15.36 (±0.5)	15.56 (±0.6)	+0.93 (±0.8)	18.84 (±2.2)	10.40 (±0.8)	−43.7 (±2.5)	1.12 (±0.1)	0.70 (±0.04)
4 walking legs (CI)	10	16.28 (±0.6)	16.74 (±0.6)	+0.87 (±0.2)	23.92 (±1.4)	23.89 (±1.3)	−0.11 (±0.4)	1.50 (±0.03)	1.50 (±0.03)
3 walking legs + cheliped	14	14.88 (±0.4)	14.91 (±0.4)	+0.58 (±0.5)	21.02 (±0.2)	10.98 (±0.7)	−45.6 (±4.1)	1.38 (±0.1)	0.71 (±0.02)
ES	39	15.76 (±0.2)	17.53 (±0.3)	+11.1 (±0.8)	22.78 (±0.6)	23.69 (±0.7)	+3.6 (±0.6)	1.49 (±0.04)	1.40 (±0.04)
ES (subsequent MA)	12	15.90 (±0.4)	17.01 (±0.4)	+7.4 (±1.4)	22.52 (±1.4)	5.82 (±0.3)	−73.3 (±2.6)	1.31 (±0.06)	0.34 (±0.02)

Abbreviations are as in Table II.

* Measuring Error = 0.56%.

TABLE VI

Changes in cheliped/carapace ratios (C/C) following three successive ecdyses.

	Mean cheliped/carapace ratios (±SEM)					
	Following ecdyses no:					
	1		2		3	
Limbs missing:	C/C ratio	Sample size (n)	C/C ratio	Saple size (n)	C/C ratio	Sample size (n)
1 Walking leg	1.43 (±0.02)	43	1.35 (±0.03)	23	1.31 (±0.04)	7
8 Walking legs (MA-CI)	1.41 (±0.04)	14	1.36 (±0.03)	3	1.34 (±0.07)	3
1 Cheliped	0.69 (±0.02)	13	0.83 (±0.02)	9	1.04 (±0.05)	6
3 Legs + cheliped	0.71 (±0.02)	12	0.90 (±0.04)	6	1.10 (±0.02)	6
7 Legs + cheliped (MA)	0.69 (±0.01)	40	0.82 (±0.02)	27	0.97 (±0.04)	10

nificantly higher than the ER of R_3 limb buds from control crabs (Table II). However, if MA occurs during the early proecdysial period (D_0), the ER of the bud is no different from that of the controls (Table II).

The final R-values of R_3 limb buds from MA crabs are the same as the final R_3 values for the controls (Fig. 3 and Table II). Yet, the post-ecdysial size of newly regenerated limbs is considerably smaller than the size of control limbs (Table VII). A non-regenerated walking leg has an average of 2.6 mg of protein, and a regenerated R_3 has 1.0 mg of protein. However, the ratio of the total amount of protein/volume (= propus length^3) is the same in newly regenerated walking legs as in non-regenerated legs (Table VII).

Carapace growth. Following a multiple autotomy-induced ecdysis, the amount of growth (expressed as increase in carapace width) is significantly reduced when compared to controls (Table V).

Crabs that regenerate eight walking legs (MA-CI) increase only 0.12% in carapace width. Crabs that have a heavier regeneration load (*i.e.* seven walking legs plus the cheliped = MA) actually decrease in width by 2.0%. These crabs, however, increase in size following the second post-autotomy ecdysis and continue to get larger at each succeeding ecdysis. By the end of the sixth post-autotomy cycle, MA crabs are not significantly smaller than control crabs (Figs. 1a and 1b).

Regeneration of the large cheliped can, by itself, reduce the amount of post-autotomy growth: crabs regenerating a single walking leg and a cheliped show less increase in carapace width following ecdysis than do controls (Table V).

Cheliped growth. A regenerated cheliped is always very small. The cheliped/carapace ratio of newly regenerated chelipeds is about 0.70 (Table VI). Crabs are unable to regenerate a full-sized cheliped regardless of the size of the total regeneration load (Table V). A small cheliped, however, grows at each ecdysis (Table VI) and the ratio of protein to volume is not significantly less than the ratio for non-regenerated chelipeds (Table VII). Crabs that have lost eight walking legs but not the cheliped are able to maintain the growth of the cheliped at each ecdysis (Tables V and VI). These crabs do not appear to regenerate walking legs at the expense of the cheliped. The percent size increase of the cheliped of these crabs is the same as the percent increases of controls. The regeneration of eight walking legs is accomplished at the expense of carapace growth and not cheliped growth (Table V).

TABLE VII

Mean (±SEM) water and protein content of chelipeds and walking legs following variously induced ecdyses.

Limbs: Type of induced molt	Sample size (n)	% H_2O (of wet weight) (±SEM)	% Protein		Mean total amount of protein (mg) (±SEM)	Mean length of propus (cm) (±SEM)	Mean ratio of total amount of protein/cm^3 (= cube of propus length) (±SEM)
			of wet weight (±SEM)	of dry weight (±SEM)			
Chelipeds:							
1) Controls	10	50.3 (±0.30)	7.6 (±0.5)	15.2 (±1.0)	32.2 (±8.6)	2.84 (±0.10)	1.75 (±0.50)
2) MA	10	69.6 (±2.5)	2.5 (±0.3)	6.2 (±1.0)	1.8 (±0.4)	1.10 (±0.09)	1.40 (±0.21)
3) ES	6	81.7 (±2.2)	1.0 (±0.1)	5.3 (±0.4)	6.7 (±0.7)	2.73 (±0.06)	0.32 (±0.12)
4) ES + MA	3	80.9 (±4.6)	2.0 (±0.5)	10.9 (±2.9)	0.34 (±0.1)	0.60 (±0.04)	1.47 (±0.39)
Walking legs:							
1) Controls	27	54.2 (±0.6)	8.8 (±1.2)	12.3 (±4.7)	2.6 (±1.0)	0.89 (±0.20)	5.97 (±1.2)
2) MA	11	68.5 (±4.5)	6.0 (±1.0)	14.5 (±4.4)	1.0 (±0.4)	0.60 (±0.06)	5.15 (±2.2)

Abbreviations are as in Table II.

A large, non-regenerated cheliped (C/C ratio = 1.66) contains an average of 32.2 mg of extractable protein (Table VII), whereas a regenerated cheliped (average C/C ratio = 0.81) has an average of 1.8 mg of protein. When a crab loses approximately 50 mg of protein (32.2 mg of cheliped protein and 18–20 mg of walking leg protein) through multiple autotomy, it regenerates only 8–9 mg of protein (approximately 1.8 mg of cheliped protein and 7–8 mg of walking leg protein).

Growth patterns following eyestalk removal

Regeneration of walking legs. Figure 3 illustrates an averaged growth curve for regenerating R_3 limb buds from eyestalkless crabs (crosses, dashed line). Rapid proecdysial limb bud growth begins soon after eyestalk removal. The growth curve for this R_3 is parallel to, but ahead of, the curve for control crabs. The R_3 limb bud of an eyestalkless crab (like the limb bud of a MA crab) has an exaggerated period of no growth or terminal plateau at the end of the proecdysial period prior to ecdysis.

Growth of an R_3 from an eyestalkless crab can be inhibited during C_4 or D_0 by multiple autotomy (Fig. 3, crossed arrow). The inhibition lasts until the newly autotomized papillae emerge, then growth of all limb buds continues at ER's comparable to other eyestalkless crabs. These crabs enter terminal plateau at R_3 values significantly lower than the final R_3 values of intact controls and eyestalkless (but otherwise untreated) crabs (Fig. 3).

Frequently, when eyestalks are removed at the same time as autotomy, the limb bud papilla will not emerge and the crab will molt without any regeneration. In most of the experiments reported here, the R_3 limb papillae were allowed to emerge prior to eyestalk removal. In about 25% of the experimental crabs, eyestalk removal did not cause limb bud growth or ecdysis. These unresponsive crabs remained alive for considerable lengths of time, then died. They generally died prior to the ecdysis of the other eyestalkless crabs.

Carapace growth. Eyestalk removal results in an 11.1% increase in carapace width (Table V). This increase is reduced to 7.4% if eyestalk removal is followed by multiple autotomy of seven walking legs plus the cheliped (Table V).

Cheliped growth. Chelipeds from recently molted, eyestalkless crabs have the same linear dimensions as do the chelipeds from control crabs (Table VII). However, the chelipeds from eyestalkless crabs contain relatively less protein and more water than do the chelipeds from controls, and the ratio of the amount of protein to cheliped volume is significantly less than controls (Table VII). When the cheliped and several walking legs are autotomized from an eyestalkless crab, the regenerated cheliped is even smaller (C/C = 0.34) and contains much less protein. The protein to volume ratios in these claws, however, are similar to the controls (Table VII).

Discussion

When male specimens of *Uca pugilator* are kept in the laboratory in constant environmental conditions (23°C, 12 hours light/day, private boxes, and oatmeal once per week) these crabs will molt and grow. The intermolt cycles of these animals are extremely variable. The crabs molt independently of one another and intermolt cycle durations vary dramatically from crab to crab and from cycle to cycle (in intact control crabs lacking one walking leg). If *Uca* are held in the lab in constant conditions for several months, there is a reduction in the mean of the molt cycle due to a reduction in the number of extremely long intermolt periods. The mean of these later intermolt cycles drops to about 70 days, but the unpredictable and variable molting patterns for individual crabs remain unchanged.

The crabs used in these experiments were collected from populations of crabs in Florida. The climate in Florida is probably less of a limiting factor to food getting and reproduction than in more temperate regions. Environmental clues serve to synchronize feeding, reproductive, and molting activities of some populations. Since natural populations of *Uca* molt in burrows (away from other members of the population) and females copulate in a hardened, intermolt stage (rather than being restricted to the shorter and softer post-molt stage) there would be no obvious survival or reproductive advantage for the members of the population to molt in synchrony (as do some of the aquatic crabs and shrimps). It is not surprising, therefore, that external clues seem to be less important in controlling intermolt cycles in Florida populations of *Uca* than has been reported for other crustaceans (Bliss and Boyer, 1964; Weis, 1976). Crane (1975) has suggested that much of the ritualistic intermale combat and courting behavior observed in populations of *Uca* in the field, serves to synchronize certain group activities. The vast differences in intermolt cycle durations reported here may be due, in part, to the fact that these experimental crabs were held in individual boxes. Crabs held apart are deprived of any social synchronization.

Although individual crabs held in constant conditions continue to molt independently of one another, they can be induced (by multiple autotomy and eyestalk removal) to enter proecdysis and molt in concert. However, the two induced proecdyses are very different: while MA and MA-CI seem to reset a highly controlled and biphasic program, eyestalk removal appears to simply remove endogenous inhibitory mechanisms (that in control animals are withheld only during late proecdysis).

The response to multiple autotomy in *Uca* is divided into two distinct phases. The first phase consists of a physiological resetting. In *Uca*, the "reset event" is (1) independent of the eyestalks; (2) inhibitory to proecdysis; and (3) the initial response to autotomy. Skinner and Graham (1972) suggested that multiple autotomy in crabs resets the entire intermolt cycle. In *Uca*, this does not seem to be the case. It appears that the reset effect of multiple autotomy is independent of the effect of multiple autotomy upon the duration of the subsequent proecdysial period. The number of days from MA (or MA-CI) to ecdysis is consistent regardless of whether MA occurs during C_4 or early D (Table II). But when MA occurs in eyestalkless animals, only the reset effect is observed. MA seems to have no effect on the proecdysial program when eyestalks are missing.

In *Uca*, autotomy-induced resetting allows for the emergence and early growth of autotomized limb buds. Adiyodi (1972) has shown that the earliest phase of regeneration (limb bud emergence and basal growth) in the crab *Paratelphusa* is characterized by extensive mitotic activity and is different from the actual proecdysial growth phase which is characterized by increased cell size rather than number. Limb bud emergence and basal growth are independent of proecdysis and are inhibited if autotomy occurs during the later stages of D (Bliss, 1956; Passano and Jyssum, 1963; Hopkins, *et al.*, 1979). Thus, when a limb is lost, it is necessary to establish the internal physiological conditions that will allow for the mitotic events of blastema organization and limb bud papillae emergence. If the function of the reset event is to allow blastema organization and early bud growth, then the reset event is not limited to multiple autotomy. The loss of a second walking leg during C_4 has a profound effect on the growth of the previously autotomized limb bud. The basal growth of the first limb bud is inhibited until the emergence of the second limb papilla. Both of these limb buds will then proceed through basal growth simultaneously. The duration from autotomy until emergence of the second papilla is significantly shorter than the time for emergence of the first limb. The simultaneous

loss of two limbs during C_4 hastens the emergence of both limb papillae (Table III). Autotomy of two limbs has a reset effect that is less than the effect of autotomy of four limbs or of MA.

The resetting event that allows for emergence of the blastema also seems to have an effect on the time that it takes the animal to reach proecdysis. There is a decrease in the number of days from autotomy to ecdysis with increasing numbers of limbs removed. Thus, there is a cumulative effect of limb loss upon the onset of proecdysial program in *Uca*. Each limb adds to the overall effect. Fingerman and Fingerman (1974) have reported in female *Uca pugilator*, an increase in molting rate (expressed as percent ecdysis/time) with increased numbers of limbs removed. Weis (1977b) reported that multiple autotomy during early proecdysis (R_1 value of 10) accelerated the growth of the original R_1 and hastened the onset of ecdysis in *Uca*. She also reported that autotomy of five or more limbs had a greater acceleratory effect than autotomy of two limbs. In describing the effects of limb loss on molt cycle in the cockroach, *Blattella*, Kunkel (1977) suggested that there is an independent signal from each regenerating limb with an average delay message programmed for each autotomized limb in the hemiganglion serving that limb. A similar model may be applicable to *Uca*, with each limb having an individual message and the final effect being the sum of those messages.

The extremely large cheliped of *Uca* has a greater resetting effect than does a single walking leg. Emergence of limb papillae in response to autotomy of four walking legs lags behind limb papillae emergence in response to loss of three walking legs and the cheliped. Also, autotomy of eight walking legs is less effective in causing a reset event in late proecdysis than is autotomy of seven walking legs plus the cheliped. These results differ from those reported for the tropical land crab, *Gecarcinus lateralis* (Skinner and Graham, 1972). In *Gecarcinus*, loss of a cheliped was no more effective than loss of a walking leg in inducing proecdysis. The large cheliped of *Uca*, however, is relatively much larger than either of the chelipeds of *Gecarcinus* and may play a more important role in the social and reproductive behavior of *Uca* than do the two chelipeds of *Gecarcinus*. Therefore, there may be a greater advantage to *Uca* to preferentially regenerate the cheliped.

The second phase of an autotomy-induced cycle is the actual growth phase of "proecdysial program." This program is (1) normally under the control of the eyestalks; and (2) disrupted by the reset event. The proecdysial duration of crabs missing eight walking legs (MA-CI) is the same as that of crabs missing their eyestalks and of control crabs (25 to 27 days). This is a significantly shorter duration than the duration from MA to ecdysis in eyed crabs (33 days). If 25–27 days represents the shortest proecdysial duration, then loss of the cheliped must exert some inhibitory control over the onset or duration of the proecdysial program. This inhibitory control is mediated through the eyestalks because MA of eyestalkless crabs resets but does not affect the proecdysial program. Likewise, MA during D_1 in intact crabs resets but does not affect the subsequent proecdysial program. Thus, in crabs with minimal (or no) eyestalk controls, MA can only initiate the reset event and has no control over the proecdysial program.

Eyestalk removal in *Uca* does not always result in regeneration and ecdysis. Up to 25% of destalked *Uca* do not respond to eyestalk removal. Charmantier-Daures (1976) reported that during stage C_4, eyestalk removal in the crab, *Pachygrapsus*, induced regeneration in only 50% of the crabs. Perhaps, these unresponsive crabs are physiologically inadequate to initiate the processes that lead to ecdysis. Unlike the crab *Gecarcinus*, eyestalkless *Uca* do not always die at or before molt. About 20% of eyestalkless *Uca* live through two ecdyses and the length of the second intermolt is virtually the same as the first intermolt duration.

It has been proposed that the effects which follow autotomy in crustaceans are due to the severance of a critical number of leg nerves (Skinner and Graham, 1972; Bittner and Kopanda, 1973). This "severed nerve hypothesis" would not, however, account for the fact that in *Uca* autotomy of the cheliped has a greater effect than autotomy of a single leg. Nor could it account for the fact that the duration of the second post-autotomy intermolt cycle is significantly shorter than the comparable intermolt cycle of the controls. (Charmantier-Daures, 1976, observed a similar effect in the crab, *Pachygrapsus*.) These facts suggest that a message with qualitative and quantitative information about the limb is conveyed to the CNS and the message is not merely an on/off signal as suggested by the severed nerve hypothesis. Newly regenerated limbs are smaller after molt than non-regenerated limbs (see below) and slight injuries may occur to the new limbs during the extremely difficult task of getting out of an old exoskeleton with a minimum number of limbs and efficiency. Minor injuries and/or small limb size may alter or modify the messages sent back to the CNS by the intact limbs. The "program" may also respond to sensory input: smaller, newly regenerated limbs may not have as many sensory structures as non-regenerated limbs.

The effects of MA (and MA-CI) are evident in the growth rates of the regenerating limb buds. MA during intermolt speeds the ER's of the resulting limb buds. During mid-proecdysis, the rates of growth are unaffected and in late proecdysis the overall rates of limb bud growth are slowed. The final size of the regenerated limb bud does not appear to be affected by speeding or slowing the growth rates. The final size of the limb buds are the same for limb buds that have regenerated slowly and buds that have regenerated quickly.

In *Uca*, ecdysis does not always result in an increase in carapace size (see also Guyselman, 1953; Weis, 1976). Ecdysis may take place solely as a means of regenerating missing limbs, and sometimes regeneration may take place at the expense of general body growth. Under the holding conditions described here, crabs that regenerate more than four legs possess a new carapace that is no larger and sometimes smaller than the one shed. There is a relationship between regeneration load and degree of growth (or no growth) observed in the post-molt carapace. Fingerman and Fingerman (1974) reported that intact female *Uca* regenerating eight walking legs showed less growth than intact crabs missing only one limb, but they did not report any loss of carapace size. The new exoskeleton of a post-ecdysial crab is initially expanded with water taken up and stored during proecdysis (Bliss and Boyer, 1964) and during post-molt the fluid is replaced with protein (Skinner, 1966). Perhaps the volume of water taken up during proecdysis is the same whether the crab is or is not regenerating limbs. During post-molt, then, an MA crab must use that volume of water to expand not only the new exoskeleton carapace but also the newly regenerated cheliped and all of the new walking legs. The reduction in carapace size (or lack of increase in size) might, therefore, be due to insufficient water uptake during proecdysis.

The failure to increase in size at ecdysis is not due to the truncated proecdysis. Eyestalkless crabs have the briefest proecdysial duration, yet eyestalkless crabs have the largest post-ecdysial increase in carapace size. MA reduces the post-molt increase in size of eyestalkless crabs. If the increase in carapace size in eyestalkless animals is due to increased water uptake, then MA may block the increase in carapace size in much the same way that it may block the increase in intact crabs.

Intact control crabs do not always have a terminal plateau at the end of the proecdysial period. Terminal plateau (a period of no growth preceding ecdysis) occurs consistently in eyestalkless and, to a lesser extent, in MA and MA-CI crabs. Crabs missing seven or eight limbs show some terminal plateau, but less terminal

plateau is evident in crabs missing fewer limbs. Perhaps regeneration becomes uncoupled from other proecdysial events in those crabs that have an exaggerated terminal plateau. The fact that eyestalkless crabs (with subsequent MA) have a terminal plateau at R-values that are significantly lower than in eyestalkless crabs suggests that terminal plateau is not due to limb buds having reached maximal size, but rather is due to physiological conditions at the end of proecdysis that are inhibitory to further growth of the limb buds. At ecdysis, eyestalkless crabs have buds that are the same size as the limb buds of intact crabs at ecdysis. The fact that these buds are no smaller than other buds is unexpected in light of the extreme differences found in size and protein content of the post-ecdysial limbs.

It has been reported in other crabs that post-molt regenerated limbs are smaller than post-molt non-regenerated limbs (Skinner and Graham, 1972; Charmantier-Daures, 1976). This is also true in *Uca*. Fingerman and Fingerman (1974) and Weis (1976) also reported that post-molt walking legs were smaller in MA *Uca*. Newly regenerated legs are 32% smaller than non-regenerated legs and contain 62% less protein. In *Uca* a regenerated cheliped is much smaller. Newly regenerated chelipeds increase in size with each succeeding ecdysis. The chelipeds increase 28% at the second post-autotomy ecdysis and continue to increase at each ecdysis. Due to the high mortality rate for MA crabs, it was never observed whether the regenerated chelipeds ever regain their former dimensions.

Under the holding conditions described above, *Uca* is capable of *de novo* synthesis of only 9 mg of protein (regardless of how many limbs were lost through autotomy). This amount of protein is much less than the amount the crab *Gecarcinus* is capable of regenerating (Skinner and Graham, 1972). However, this difference may be due to the fact that *Gecarcinus* is a considerably larger crab.

Skinner (1966) reported that the amount of muscle per cheliped in *Gecarcinus* was lowest during the first few days after ecdysis and the maximal growth of the chelipeds (in terms of incorporation of ^{14}C-leucine into protein) occurred during post-molt. The post-molt size of an unregenerated cheliped from an eyestalkless *Uca* has the same linear dimensions as the unregenerated cheliped from an eyed control crab. However, the ratio of protein to volume of the cheliped from the eyestalkless crab is greatly reduced. These chelipeds from eyestalkless crabs grow over 5% in linear dimensions following ecdysis but contain much less protein. This is probably due to the fact that these eyestalkless crabs have little or no post-molt, but rather pass very quickly from ecdysis into a new proecdysial period. Thus, eyestalkless crabs have less "down time" in which muscle protein can be synthesized to replace muscle protein autolysed during proecdysis. On the other hand, eyestalkless crabs that are subsequently autotomized (including the cheliped) are sufficiently inhibited by the resetting action of autotomy that they can regenerate the cheliped. The period of regeneration is so short, however, that the linear dimensions of the newly regenerated cheliped are only half the dimensions of chelipeds regenerated by intact crabs. Perhaps the physiological conditions of proecdysis are inhibitory to protein synthesis, or the autolysis of muscle that occurs during proecdysis is so extensive that it somehow overrides most synthetic efforts.

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